Characterization of Pentasaccharide Glycosides from the Roots of *Ipomoea arborescens*

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Ten new pentasaccharide glycosides, arboresins 1−6 (**1**−**6**) and murucins 6−9 (**8**−**11**), along with five known glycolipids, were isolated from the roots of *Ipomoea arborescens*, and their structures were elucidated by spectroscopic and chemical methods. Compounds **1**−**6** and **8**−**11** were evaluated for cytotoxicity against a small panel of cancer cell lines.

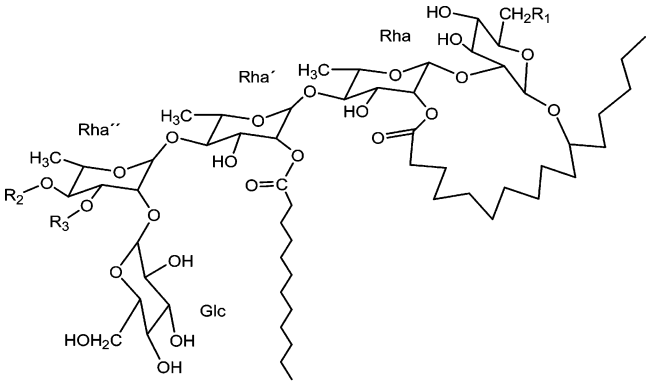
Ipomoea arborescens Hum. et Bonpl. (Convolvulaceae) is a tree that usually flowers and fruits between November and April. This plant inhabits open thorn forests, oak savannas, and deciduous forests and is distributed throughout western Mexico. This species is known in several states of Mexico as "cazahuate" and "palo bobo". Some communities use an aqueous infusion of the bark and other herbs against snake and scorpion bites and to prevent hair loss. An aqueous infusion of the leaves has been used as an antiinflammatory and to treat stomachache.[1] Perez Amador et al.[2] detected the presence of resin glycosides in leaves of *I. arborescens* but without divulging any chemical structures. In our continuing investigation on secondary metabolites with biological activity from *Ipomoea* species, we have studied the resin glycosidic content of the roots of *I. arborescens*. We report herein on the isolation and characterization of 10 new pentasaccharides of jalapinolic acid, arboresins 1−6 (**1**−**6**) and murucins 6−9 (**8**−**11**), and five known glycolipids, murucins 1−5, from *I. arborescens*.

Results and Discussion

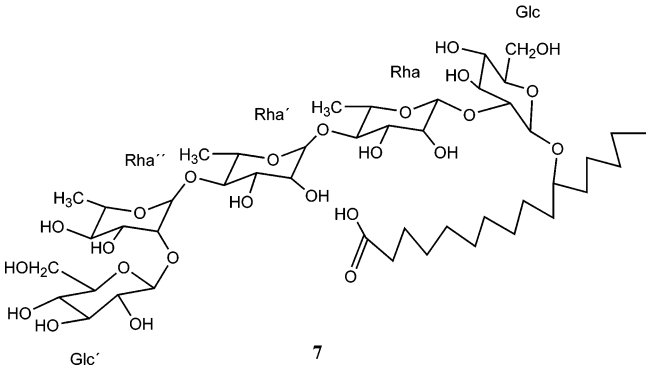
The roots of *I. arborescens* were dried, pulverized, and macerated in chloroform, and the extract was fractionated by column chromatography on silica gel, leading to the separation of two chromatographic fractions. Both crude chromatographic fractions exhibited low cytotoxic activity for a cancer cell line (OVCAR-5, ED_{50} 4.5 μg/mL) and were subjected to preparative HPLC in the reversed-phase mode, with compounds **1**−**6** and **8**−**11**, respectively, being isolated by repetitive chromatography.

The less polar chromatographic fraction was hydrolyzed in an aqueous/ethanolic acid medium, producing an organic fraction together with a water-soluble mixture of carbohydrates. Analysis of the organic fraction by GC-MS permitted the identification of acetic, propanoic, butanoic, 2-methylbutanoic, 3-hydroxy-2-methylbutanoic (nilic), dodecanoic, and 11-hydroxyhexadecanoic ethyl esters by comparison with the mass spectra and retention times of the ethyl esters of authentic samples. HPLC and GC-MS analysis of the carbohydrates present in the aqueous phase allowed the identification of rhamnose and glucose.

Basic hydrolysis of the less polar chromatographic fraction produced an organic acid fraction and a water-soluble glycosidic acid derivative (**7**). Arboresinic acid (**7**) gave a quasi-molecular ion at *m/z* 1057 $[M + Na]^+$ in the positive-ion FABMS. A deprotonated glycosidic acid $[M - H]^-$ peak was observed in the negative-ion HRESIMS (*m/z* 1033.7730), consistent with a molecular formula of $C_{46}H_{82}O_{25}$. Negative-ion FABMS of **7** showed a deprotonated molecular ion at *m/z* 1033 $[M - H]^-$ and other significant peaks at *m/z* 871 [*m/z* 1033−162 $(C_6H_{10}O_5)]^-$, 725 [*m/z*



	R_1	R_2	R_3
1	OH	H	Niloyl
2	OH	Acetyl	Niloyl
3	OH	Propanoyl	Niloyl
4	OH	Butanoyl	Niloyl
5	OH	2-methylbutanoyl	Niloyl
6	OH	Tigloyl	Niloyl
8	H	Acetyl	Niloyl
9	H	2-butenoyl	Niloyl
10	H	Tigloyl	Niloyl
11	H	H	H



7

871−146 $(C_6H_{10}O_4)]^-$, 579 [*m/z* 725−146 $(C_6H_{10}O_4)]^-$, 433 [*m/z* 579−146 $(C_6H_{10}O_4)]^-$, and 271 [*m/z* 433−162 $(C_6H_{10}O_5)]^-$. The sugar units in the ^{1}H NMR spectrum of **7** (Table 1) showed three doublet methyl signals of 6-deoxyhexose units. The ^{13}C NMR spectrum of **7** (Table 1) exhibited five anomeric signals, demonstrating that part of the molecule is a pentasaccharide. The HMQC spectrum of **7** indicated that anomeric carbons at 103.1, 103.0, 102.3, 101.9, and 101.2 ppm were correlated with the anomeric protons at 5.05 (d, $J = 7.7$ Hz), 5.18 (d, $J = 7.6$ Hz), 5.38 (d, $J = 1.6$ Hz), 5.24 (d, $J = 1.5$ Hz), and 5.49 (d, $J = 1.5$ Hz) ppm,

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Table 1. ^{1}H and ^{13}C NMR Data for Arboresinic Acid (**7**) (D_2O, δ ppm, J in Hz)

position	δ_H	δ_C
Glc[a]		
1	5.18 d (7.6)	103.0
2	3.50*[b]	79.3
3	3.63*	78.5
4	3.02 dd (9.0, 9.5)	77.3
5	3.30 ddd (2.5, 5.0, 9.0)	72.7
6	3.50*	63.4
6′	3.85 dd (2.5, 12.0)	
Rha		
1	5.24 d (1.5)	101.9
2	3.92 dd (3.3, 1.3)	71.9
3	4.01 dd (9.1, 3.3)	72.0
4	3.60*	72.2
5	4.22 dd (6.2, 9.2)	67.9
6	1.24 d (6.2)	19.1
Rha′		
1	5.49 d (1.5)	101.2
2	4.02 dd (3.5, 1.5)	70.0
3	4.20 dd (9.0, 3.5)	71.4
4	3.75*	71.1
5	4.28 dd (6.0, 8.9)	68.1
6	1.01 d (6.0)	18.5
Rha″		
1	5.38 d (1.6)	102.3
2	4.11 dd (3.4, 1.4)	70.9
3	4.28 dd (8.9, 3.4)	71.7
4	3.65*	72.0
5	4.42 dd (6.0, 9.0)	68.7
6	1.15 d (6.0)	19.0
Glc′		
1	5.05 d (7.7)	103.1
2	3.45 dd (9.1, 7.6)	78.1
3	3.69*	79.1
4	3.10 dd (9.0, 9.0)	72.9
5	3.25 ddd (2.4, 4.9, 9.0)	78.0
6	3.60*	63.7
6′	3.90 dd (2.4, 12.1)	
Jal		
1		179.1
2	2.38 t (6.7)	36.5
11	3.75*	83.0
16	1.00 t (7.0)	15.0

[a] Abbreviations: Qui = quinovopyranosyl, Glc = glucopyranosyl, Rha = rhamnopyranosyl, and Jal = 11-hydroxyhexadecanoyl. [b] Chemical shifts marked with an asterisk (*) indicate overlapped signals.

respectively. A combination of one- and two-dimensional ^{1}H NMR techniques allowed all protons to be assigned sequentially within each saccharide system, leading to the identification of two glucopyranosyl and three rhamnopyranosyl units as the monosaccharides present in **7**. The anomeric configurations for the sugar moieties were assigned as β for glucopyranosyl and α for rhamnopyranosyl from their coupling constants of 7.7 and 1.6 Hz, respectively. All the resonances in the ^{13}C NMR spectrum were assigned from the HMQC NMR spectrum. The connectivities between sugar moieties were determined from the following HMBC correlations: C-2 (79.3 ppm) of glucose with H-1 (5.25 ppm) of rhamnose; C-4 (82.4 ppm) of rhamose with H-1 (5.45 ppm) of rhamnose′; C-4 (81.3 ppm) of rhamnose′ with H-1 (5.35 ppm) of rhamnose″; and C-2 (70.9 ppm) of rhamnose″ with H-1 (5.05 ppm) of glucose′, corroborating that the oligosaccharide chain is linear. The position of the jalapinolic acid unit was determined by the correlation between jalapinolic acid H-11 (3.75 ppm) and glucose H-1 (4.40 ppm) in the ROESY NMR spectrum. Accordingly, the structure of arboresinic acid (**7**) was assigned as (11*S*)-hydroxyhexadecanoic acid 11-*O*-β-D-glucopyranosyl-(2→1)-*O*-α-L-rhamnopyranosyl-(4→1)-*O*-α-L-rhamnopyranosyl-(4→1)-*O*-α-L-rhamnopyranosyl-(2→1)-*O*-β-D-glucopyranoside. The structure of arboresinic acid is similar to that of operculinic acid, with the only difference being in the position of glucose′.[3]

Table 2. ^{13}C NMR Data for Compounds **1**−**6** (100 MHz, CD_3OD)

carbon	**1**	**2**	**3**	**4**	**5**	**6**
Glc						
1	105.4	105.4	105.3	105.4	105.4	105.3
2	80.1	80.0	80.0	80.1	79.9	80.0
3	73.8	73.7	73.8	73.7	73.6	73.5
4	71.4	71.3	71.4	71.4	71.3	71.4
5	73.3	73.3	73.3	73.2	73.1	73.3
6	62.7	62.8	62.8	63.0	62.9	62.8
Rha						
1	98.9	99.0	98.9	98.8	98.8	98.9
2	84.1	84.1	84.1	84.1	84.1	84.1
3	70.1	70.1	70.2	70.1	70.1	70.1
4	72.0	72.0	72.0	72.0	72.0	71.9
5	69.2	69.3	69.2	69.2	69.2	69.2
6	19.2	19.3	19.3	19.3	19.3	19.3
Rha′						
1	100.2	100.2	100.2	100.2	100.2	100.2
2	80.9	80.8	80.8	80.8	80.8	80.7
3	73.7	73.8	73.7	73.7	73.7	73.7
4	73.1	73.1	73.1	73.1	73.1	73.1
5	69.1	69.0	69.0	69.0	69.0	69.0
6	18.8	18.8	18.8	18.8	18.8	18.8
Rha″						
1	103.2	103.3	103.2	103.3	103.3	103.2
2	72.2	72.2	72.2	72.2	72.2	72.2
3	80.3	80.3	80.3	80.3	80.3	80.3
4	72.5	82.4	82.4	82.5	82.5	82.5
5	68.6	68.6	68.6	68.6	68.6	68.6
6	17.8	17.8	17.9	17.8	17.8	17.8
Glc′						
1	105.5	105.5	105.5	105.5	105.5	105.5
2	75.3	75.3	75.3	75.3	75.3	75.2
3	78.3	78.3	78.3	78.2	78.2	78.3
4	71.6	71.5	71.5	71.5	71.5	71.5
5	78.1	78.2	78.1	78.1	78.1	78.1
6	63.1	63.1	63.2	63.2	63.2	63.2
Jal-1	175.0	175.0	174.9	175.0	175.0	175.1
2	34.8	34.8	34.8	34.8	34.8	34.8
11	84.0	84.0	84.0	84.0	84.0	84.0
16	14.4	14.4	14.4	14.4	14.4	14.4
dodeca-1	175.1	175.1	175.0	175.1	175.0	175.0
2	35.1	35.0	35.0	35.0	35.0	35.0
12	23.1	23.0	23.0	23.0	23.0	23.0
ac 1		172.4				
2		41.0				
propa-1			178.3			
2			34.9			
3			20.1			
buta-1				178.2		
2				42.1		
3				17.9		
4				19.5		
mba-1					178.3	
2					40.8	
3					28.7	
4					19.0	
nl-1						178.0
2						48.5
3						69.8
4						20.1
nl′-1	178.1	178.0	177.9	177.9	178.0	177.9
2	48.3	48.2	48.2	48.3	48.2	48.2
3	69.9	69.8	69.9	69.8	69.9	70.0
4	20.0	20.1	20.1	20.0	20.1	20.2

[a] Abbreviations: Qui = quinovopyranosyl, Glc = glucopyranosyl, Rha = rhamnopyranosyl, dodeca = *n*-dodecanoyl, ac = acetyl, propa = propanoyl, buta = butanoyl, mba = 2-methylbutanoyl, nl = 3-hydroxy-2-methylbutanoyl, and Jal = 11-hydroxyhexadecanoyl.

Arboresin 1 (**1**) gave a quasi-molecular ion at *m*/*z* 1321 $[M + Na]^+$ in the positive-ion FABMS. An accurate mass measurement of the deprotonated molecule $[M - H]^-$ in the negative-ion HRESIMS gave *m*/*z* 1297.8365 $[M - H]^-$, consistent with the molecular formula, $C_{63}H_{110}O_{27}$. The negative-ion FABMS showed fragment peaks at *m*/*z* 1297 $[M - H]^-$, 1115 [*m*/*z* 1297−182

Table 3. ^{1}H NMR Data for Compounds **1**−**6** (CD_3OD, δ ppm, J in Hz)[a]

position	1	2	3	4	5	6
Glc 1	4.41 d (7.0)	4.43 d (7.2)	4.41 d (7.1)	4.42 d (7.0)	4.44 d (7.9)	4.44 d (7.9)
2	3.20 dd (9.0, 7.0)	3.24 dd (9.0, 7.2)	3.23 dd (8.9, 7.1)	3.23 dd (9.1, 7.0)	3.22 dd (9.0, 7.9)	3.22 dd (9.0, 7.9)
3	3.30 dd (9.0, 9.0)	3.32 dd (8.9, 9.0)	3.33 dd (9.0, 8.9)	3.52 dd (9.0, 9.0)	3.52 dd (8.9, 9.1)	3.52 dd (8.9, 9.1)
4	3.58*	3.60*	3.60*	3.61*	3.60*	3.60*
5	3.20 dd (9.0, 8.9)	3.20 dd (8.9, 8.9)	3.21 dd (9.0, 9.0)	3.20 (8.9, 9.0)	3.21 dd (8.9, 9.0)	3.21 dd (8.9, 9.0)
6	3.60*	3.61*	3.61*	3.61*	3.62 d (7.1)	3.62 d (7.1)
6′	3.85*	3.85*	3.87*	3.86*	3.84*	3.84*
Rha						
1	5.04 d (1.7)	5.02 d (1.8)	5.03 (1.7)	5.02 d (1.7)	5.03 d (1.8)	5.03 d (1.8)
2	5.10 dd (3.6, 1.7)	5.16 dd (3.7, 1.8)	5.13 dd (3.6, 1.7)	5.16 dd (3.8, 1.7)	5.16 dd (3.7, 1.8)	5.16 dd (3.7, 1.8)
3	4.22 dd (9.3, 3.6)	4.20 dd (9.1,3.7)	4.21 dd (9.2, 3.6)	4.22 dd (9.3, 3.8)	4.21 dd (9.2, 3.7)	4.21 dd (9.2, 3.7)
4	3.46 dd (9.0, 9.3)	3.44 dd (9.1, 9.1)	3.47 dd (9.0, 9.2)	3.45 dd (9.1, 9.3)	3.46 dd (9.2, 9.2)	3.46 dd (9.2, 9.2)
5	3.86 *	3.87 *	3.88 *	3.89 *	3.87 *	3.87 *
6	1.28 d (6.6)	1.27 d (6.5)	1.28 d (6.5)	1.29 d (6.6)	1.28 d (6.5)	1.28 d (6.5)
Rha′						
1	5.08 d (1.5)	5.09 d (1.5)	5.09 d (1.5)	5.09 d (1.5)	5.10 d (1.5)	5.10 d (1.5)
2	5.52 dd (3.5, 1.5)	5.54 dd (3.4, 1.5)	5.54 dd (3.5, 1.5)	5.54 dd (3.4, 1.5)	5.57 dd (3.5, 1.5)	5.57 dd (3.5, 1.5)
3	4.06 dd (9.0, 3.5)	4.10 dd (9.1, 3.4)	4.08 dd (9.0, 3.5)	4.07 dd (9.0, 3.4)	4.07 dd (9.0, 3.5)	4.07 dd (9.0, 3.5)
4	3.66*	3.67*	3.66*	3.67*	3.67*	3.67*
5	3.86*	3.86*	3.86*	3.87*	3.86*	3.86*
6	1.30 d (6.5)	1.32 d (6.3)	1.30 d (6.3)	1.29 d (6.4)	1.29 d (6.4)	1.29 d (6.4)
Rha″						
1	5.27 d (1.7)	5.25 d (1.6)	5.25 d (1.7)	5.25 d (1.7)	5.26 d (1.7)	5.26 d (1.7)
2	5.06 dd (3.5, 1.7)	5.05 dd (3.5, 1.6)	5.05 dd (3.5, 1.7)	5.03 dd (3.6, 1.7)	5.05 dd (3.5, 1.7)	5.05 dd (3.5, 1.7)
3	4.74 dd (9.0, 3.5)	4.75 dd (8.9, 3.5)	4.74 dd (9.0, 3.5)	4.75 dd (9.0, 3.6)	4.74 dd (9.0, 3.5)	4.74 dd (9.0, 3.5)
4	4.93 dd (9.4, 9.0)	4.97 dd (9.2, 9.0)	4.95 dd (9.3, 9.0)	4.95 dd (9.3, 9.0)	4.95 dd (9.2, 9.0)	4.95 dd (9.2, 9.0)
5	3.84*	3.83*	3.83*	3.83*	3.83*	3.83*
6	1.14 d (6.0)	1.12 d (6.1)	1.12 d (6.0)	1.12 d (6.0)	1.12 d (6.0)	1.12 d (6.0)
Glc′						
1	4.43 d (7.1)	4.42 d (7.0)	4.42 d (7.1)	4.43 d (7.0)	4.43 d (7.0)	4.43 d (7.0)
2	3.18 dd (9.1, 7.1)	3.17 d (9.0, 7.0)	3.18 d (9.0, 7.1)	3.17 d (9.0, 7.0)	3.16 d (9.1, 7.0)	3.16 d (9.1, 7.0)
3	3.31*	3.31*	3.31*	3.31*	3.31*	3.31*
4	3.19 dd (9.0, 9.1)	3.20 dd (9.1, 9.0)	3.19 dd (9.0, 9.0)	3.20 dd (9.0, 9.1)	3.20 dd (9.0, 9.1)	3.20 dd (9.0, 9.1)
5	3.29*	3.29*	3.30*	3.30*	3.30*	3.30*
6	3.58*	3.59*	3.60*	3.60*	3.60*	3.60*
6′	3.86*	3.87*	3.87*	3.86*	3.87*	3.87*
Jal						
2a	2.31 ddd (14.0, 7.0, 3.5)	2.30 ddd (15.0, 7.5, 3.4)	2.29 ddd (15.0, 7.5, 3.4)	2.30 ddd (14.5, 7.0, 3.5)	2.30 ddd (14.0, 7.0, 3.5)	2.30 ddd (14.0, 7.0, 3.5)
2b	2.55 ddd (14.0, 7.0, 3.5)	2.56 ddd (15.0, 7.5, 3.4)	2.55 ddd (15.0, 7.5, 3.4)	2.56 ddd (14.5, 7.0, 3.5)	2.57 ddd (14.0, 7.0, 3.5)	2.57 ddd (14.0, 7.0, 3.5)
11	3.59*	3.60*	3.60*	3.60*	3.60*	3.60*
16	0.89 t (7.0)	0.88 t (7.0)	0.89 t (7.0)	0.90 t (7.0)	0.89 t (7.0)	0.89 t (7.0)
dodeca						
2	2.47 t (6.5)	2.48 t (6.7)	2.47*	2.46 t (6.7)	2.47 t (6.7)	2.47 t (6.7)
12	1.22 t (7.0)	1.23 t (7.1)	1.22 t (7.0)	1.23 t (7.1)	1.22 t (7.1)	1.22 t (7.1)
ac						
2		2.09 s				
propa						
2			2.47*			
3			1.23 t (7.1)			
buta						
2				2.50*		
4				1.10 t (7.4)		
mba						
2					2.40 t (6.4)	
4					1.09 t (7.1)	
nl						
2						2.50*
3						3.91*
nl′						
2	2.50*	2.47*	2.48*	2.48*	2.49*	2.48*
3	3.88*	3.90*	3.90*	3.90*	3.89*	3.89*

[a] Abbreviations: Glc = glucopyranosyl, Rha = rhamnopyranosyl, dodeca = *n*-dodecanoyl, ac = acetyl, propa = propanoyl, buta = butanoyl, mba = 2-methylbutanoyl, nl = 3-hydroxy-2-methylbutanoyl, and Jal = 11-hydroxyhexadecanoyl. [b] Chemical shifts marked with an asterisk (*) indicate overlapped signals.

$(C_{12}H_{22}O)]^-$, 1015 [*m*/*z* 1115−100 $(C_5H_8O_2)]^-$, 853 [*m*/*z* 1015−162 $(C_6H_{10}O_4)]^-$, 707 [*m*/*z* 853−146 $(C_6H_{10}O_4)]^-$, 561 [*m*/*z* 707−146 $(C_6H_{10}O_4)]^-$, 433 [*m*/*z* 561−128 $(C_6H_8O_3)]^-$, and 271 [*m*/*z* 433−162 $(C_6H_{10}O_5)]^-$. Basic hydrolysis of **1** afforded arboresinic (**7**), dodecanoic, and nilic acids. The ^{13}C NMR spectrum of **1** (Table 2) included three carbonyl signals and five anomeric signals. The ^{1}H NMR spectrum of **1** (Table 3) exhibited three doublet methyl signals of the three 6-deoxyhexose units, overlapped signals at 3.90 (H-3) and 2.50 ppm (H-2) of a 3-hydroxy-2-methylbutanoyl group, and two signals at 2.31 (1H, ddd) and 2.55 (1H, ddd) ppm of the nonequivalent protons of a methylene group at C-2 in the aglycon moiety, suggesting a macrocyclic lactone-type structure. Also observed were a methyl triplet signal at 1.22 ppm and a triplet-like signal for a methylene group at C-2 (2.47 ppm) of a dodecanoyl group. The position of the jalapinolic acid moiety in the oligosaccharide was determined by the correlation between jalapinolic acid

H-11 (3.62 ppm) and glucose H-1 (4.32 ppm) in a T-ROESY NMR spectrum. The HMBC NMR spectrum of **1** permitted the esterification sites to be established through the conectivities between carbonyl and ^{1}H NMR signals of the monosaccharides: the ^{13}C═O (178.0 ppm) of the 3-hydroxy-2-methylbutanoyl (niloyl) group correlated with H-4 (4.92 ppm) of rhamnose″; the ^{13}C═O (175.1 ppm) of dodecanoyl correlated with H-2 (5.51 ppm) of rhamnose′; and the ^{13}C═O (175.0 ppm) of the 11-hydroxyhexadecanoyl correlated with H-2 (5.08 ppm) of rhamnose.

Compounds **2**−**6** gave a quasi-molecular ion $[M + Na]^+$ at *m*/*z* 1363, 1377, 1391, 1405, and 1421, respectively, in the positive-ion FABMS. The molecular formulas of **2** ($C_65H_112O_28$), **3** ($C_66H_114O_28$), **4** ($C_67H_116O_28$), **5** ($C_68H_118O_28$), and **6** ($C_68H_118O_29$) were determined by their negative-ion HRESIMS. The negative-ion FABMS of **2**−**6** showed the fragment peaks $[M - H]^-$, $[(M - H)^- - (C_{12}H_{22}O)]^-$, $[(M - H)^- - (C_{12}H_{22}O)^- - (C_5H_8O_2)]^-$, and $[(M - H)^- - (C_{12}H_{22}O)^- - (C_5H_8O_2) - (acyl)]^-$, besides the common fragmentation peaks[4] produced by glycosidic cleavage of the sugar moieties. Basic hydrolysis of **2**−**6** afforded arboresinic acid (**7**) as the glycosidic acid present in these compounds.

The ^{13}C NMR spectrum of **2**−**6** (Table 2) included four carbonyl signals and five anomeric signals. The ^{1}H NMR spectrum of **2**−**6** (Table 3) exhibited three doublet methyl signals of the three 6-deoxyhexose units and two signals of the nonequivalent protons of a methylene group at C-2 in the aglycon moiety, suggesting a macrocyclic lactone-type structure. Also observed were a methyl triplet signal at 1.22 ppm and a triplet-like signal for a methylene group at C-2 (2.47 ppm) of a dodecanoyl group. The position of the jalapinolic acid moiety in the oligosaccharide core of compounds **2**−**6** was determined by the correlation between jalapinolic acid H-11 and glucose H-1 in a T-ROESY NMR spectrum. The HMBC NMR spectrum permitted the esterification sites to be established through the conectivities between carbonyl and ^{1}H NMR signals of the monosaccharides: thus for **2**−**6**, a niloyl substituent was located at C-3 of rhamnose″, a *n*-dodecanoyl residue was assigned at C-2 of rhamnose′, and the jalapinolic acid unit was esterified at C-2 of rhamnose. According to long-range correlations for **2**−**6**, an acetyl, a propanoyl, a butanoyl, a 2-methylbutanoyl, and a niloyl substituent was located at C-4 of rhamnose″, respectively.

The more polar chromatographic fraction was hydrolyzed in an aqueous/ethanolic acid medium, and the analysis of the organic fraction by GC-MS permitted the identification of acetic, propanoic, butanoic, 2-methylbutanoic, 2-methylbutenoic, 2-methyl-2-butenoic (tiglic), nilic, dodecanoic, and 11-hydroxyhexadecanoic ethyl esters by comparison with the mass spectra and retention times of the ethyl esters of authentic samples. The carbohydrates present in the aqueous phase were quinovose, rhamnose, and glucose, as identified by GC-MS and HPLC.

Basic hydrolysis of the more polar chromatographic fraction of *I. arborescens* roots produced an organic acid fraction and a water-soluble glycosidic acid derivative. The structure of the glycosidic acid was confirmed from its one- and two-dimensional NMR data as murucinic acid.[3]

The positive-ion FABMS of compound **8** gave a quasimolecular ion at *m*/*z* 1347 $[M + Na]^+$. The negative-ion HRESIMS of **8** gave a *m*/*z* value of 1323.8490 $[M - H]^-$, consistent with a molecular formula of $C_{65}H_{112}O_{27}$. The negative-ion FABMS showed fragment peaks at *m*/*z* 1323 $[M - H]^-$, 1141 [*m*/*z* 1323−182 $(C_{12}H_{22}O)]^-$, 1041 [*m*/*z* 1141−100 $(C_5H_8O_2)]^-$, 999 [*m*/*z* 1041−42 $(C_2H_2O)]^-$, 837 [*m*/*z* 999−162 $(C_6H_{10}O_4)]^-$, 691 [*m*/*z* 837−146 $(C_6H_{10}O_4)]^-$, 545 [*m*/*z* 691−146 $(C_6H_{10}O_4)]^-$, 417 [*m*/*z* 545−128 $(C_6H_8O_3)]^-$, and 271 [*m*/*z* 417−146 $(C_6H_{10}O_4)]^-$. Basic hydrolysis of **8** afforded murucinic, dodecanoic, nilic, and acetic acids. The ^{13}C NMR spectrum of **8** (Table 4) showed four carbonyl signals and five anomeric signals. The ^{1}H NMR spectrum of **8** (Table 5) demonstrated the presence of four doublet methyl signals of the 6-deoxyhexose units, a methyl singlet signal at 2.10 ppm of an acetyl group, overlapped signals at 3.90 (H-3) and 2.50 ppm (H-2) of the niloyl group, and two signals at 2.31 (1H, ddd) and 2.55 (1H, ddd) ppm of the nonequivalent protons of the methylene group at C-2 in the aglycon moiety, suggesting a macrocyclic lactone-type structure. The position of the jalapinolic acid moiety in the oligosaccharide was determined by the correlation between jalapinolic acid H-11 (3.62 ppm) and glucose H-1 (4.32 ppm) in a T-ROESY NMR spectrum. The positions of the oligosaccharide core were determined by long-range correlations in the HMBC NMR spectrum. Thus, a niloyl group was attached to C-3 of rhamnose″, an acetyl residue was located at C-4 of rhamnose″, a *n*-dodecanoyl unit was attached at C-2 of rhamnose′, and a 11-hydroxyhexadecanoyl was located at C-2 of rhamnose.

Table 4. ^{13}C NMR Data for Compounds **8**−**11** (CD_3OD)

carbon	**8**	**9**	**10**	**11**
Qui 1	105.4	105.4	105.3	105.4
2	80.1	80.0	80.0	80.1
3	73.8	73.7	73.8	73.7
4	71.4	71.3	71.4	71.4
5	73.3	73.3	73.3	73.2
6	16.9	16.8	16.8	16.8
Rha				
1	98.9	99.0	98.9	98.8
2	84.1	84.1	84.1	84.1
3	70.1	70.1	70.2	70.1
4	72.0	72.0	72.0	72.0
5	69.2	69.3	69.2	69.2
6	19.2	19.3	19.3	19.3
Rha′				
1	100.2	100.2	100.2	100.2
2	70.8	70.8	70.8	70.8
3	83.7	83.8	83.7	83.7
4	71.1	71.1	71.1	71.1
5	69.1	69.0	69.0	69.0
6	18.8	18.8	18.8	18.8
Rha″				
1	103.2	103.3	103.2	103.3
2	72.2	72.2	72.2	72.2
3	80.3	80.3	80.3	70.3
4	79.5	79.4	79.4	70.5
5	68.6	68.6	68.6	68.6
6	17.8	17.8	17.9	17.8
Glc′				
1	105.5	105.5	105.5	105.5
2	75.3	75.3	75.3	75.3
3	78.3	78.3	78.3	78.2
4	71.6	71.5	71.5	71.5
5	78.1	78.2	78.1	78.1
6	63.1	63.1	63.2	63.2
Jal				
1	175.0	175.0	174.9	175.0
2	34.8	34.8	34.8	34.8
11	84.0	84.0	84.0	84.0
16	14.4	14.4	14.0	14.0
dodeca-1	175.1	175.1	175.1	175.1
2	35.1	35.0	35.0	35.0
12	23.1	23.0	23.0	23.0
ac 1	172.5			
2	41.1			
2bute-1		178.0		
2		129.9		
3		132.2		
4		19.4		
tgl-1			172.6	
2			129.6	
3			139.1	
4			18.2	
nl-1	178.0	178.1	178.2	177.9
2	48.1	48.1	48.0	47.9
3	69.7	69.8	69.6	69.8
4	19.8	20.0	20.1	20.0

[a] Abbreviations: Qui = quinovopyranosyl, Glc = glucopyranosyl, Rha = rhamnopyranosyl, dodeca = *n*-dodecanoyl, ac = acetyl, 2bute = 2-butenoyl, tgl = 2-methyl-2-butenoyl, nl = 3-hydroxy-2-methylbutanoyl, and Jal = 11-hydroxyhexadecanoyl.

Table 5. ^{1}H NMR Data for Compounds **8**−**11′** (CD_3OD, δ ppm, J in Hz)[a]

position	8	9	10	11
Qui 1	4.31 d (7.4)	4.33 d (7.5)	4.31 d (7.7)	4.32 d (7.8)
2	3.43 dd (9.1, 7.4)	3.44 dd (9.0, 7.5)	3.43 dd (8.9, 7.7)	3.43 dd (9.0, 7.8)
3	3.50 dd (9.0, 9.1)	3.52 dd (8.9, 9.0)	3.53 dd (9.0, 8.9)	3.52 dd (9.0, 9.0)
4	3.58*	3.61*	3.62*	3.62*
5	3.60*	3.60*	3.61*	3.60*
6	1.23 d (7.0)	1.21 d (7.2)	1.22 d (7.0)	1.21 d (7.0)
Rha				
1	5.04 d (1.7)	5.02 d (1.8)	5.03 (1.7)	5.02 d (1.7)
2	5.10 dd (3.6, 1.7)	5.16 dd (3.7, 1.8)	5.13 dd (3.6, 1.7)	5.16 dd (3.8, 1.7)
3	4.22 dd (9.3, 3.6)	4.20 dd (9.1, 3.7)	4.21 dd (9.2, 3.6)	4.22 dd (9.3, 3.8)
4	3.46 dd (9.0, 9.3)	3.44 dd (9.1, 9.1)	3.47 dd (9.0, 9.2)	3.45 dd (9.1, 9.3)
5	3.86*	3.87*	3.88*	3.89*
6	1.28 d (6.6)	1.27 d (6.5)	1.28 d (6.5)	1.29 d (6.6)
Rha1				
1	5.08 d (1.5)	5.09 d (1.5)	5.09 d (1.5)	5.09 d (1.5)
2	5.52 dd (3.5, 1.5)	5.54 dd (3.4, 1.5)	5.54 dd (3.5, 1.5)	4.54 dd (3.4, 1.5)
3	5.06 dd (9.0, 3.5)	5.10 dd (9.1, 3.4)	5.08 dd (9.0, 3.5)	4.07 dd (9.0, 3.4)
4	3.66*	3.67*	3.66*	3.67*
5	3.86*	3.86*	3.86*	3.87*
6	1.30 d (6.5)	1.32 d (6.3)	1.30 d (6.3)	1.29 d (6.4)
Rha″				
1	5.27 d (1.7)	5.25 d (1.6)	5.25 d (1.7)	5.25 d (1.7)
2	4.06 dd (3.5, 1.7)	4.05 dd (3.5, 1.6)	4.05 dd (3.5, 1.7)	4.03 dd (3.6, 1.7)
3	3.74 dd (9.0, 3.5)	3.75 dd (8.9, 3.5)	3.74 dd (9.0, 3.5)	3.75 dd (9.0, 3.6)
4	4.93 dd (9.4, 9.0)	4.97 dd (9.2, 9.0)	4.95 dd (9.3, 9.0)	4.95 dd (9.3, 9.0)
5	3.84*	3.83*	3.83*	3.83*
6	1.14 d (6.0)	1.12 d (6.1)	1.12 d (6.0)	1.12 d (6.0)
Glc′				
1	4.43 d (7.1)	4.42 d (7.0)	4.42 d (7.1)	4.43 d (7.0)
2	3.18 dd (9.1, 7.1)	3.17 d (9.0, 7.0)	3.18 d (9.0, 7.1)	3.17 d (9.0, 7.0)
3	3.31*	3.31*	3.31*	3.31*
4	3.19 dd (9.0, 9.1)	3.20 dd (9.1, 9.0)	3.19 dd (9.0, 9.0)	3.20 dd (9.0, 9.1)
5	3.29*	3.29*	3.30*	3.30*
6	3.58*	3.59*	3.60*	3.60*
6′	3.86*	3.87*	3.87*	3.86*
Jal				
2a	2.31 ddd (14.0, 7.0, 3.5)	2.30 ddd (15.0, 7.5, 3.4)	2.29 ddd (15.0, 7.5, 3.4)	2.30 ddd (14.5, 7.0, 3.5)
2b	2.55 ddd (14.0, 7.0, 3.5)	2.56 ddd (15.0, 7.5, 3.4)	2.55 ddd (15.0, 7.5, 3.4)	2.56 ddd (14.5, 7.0, 3.5)
11	3.59*	3.60*	3.60*	3.65*
16	0.89 t (7.0)	0.88 t (7.0)	0.89 t (7.0)	0.90 t (7.0)
dodeca				
2	2.47 t (6.5)	2.48 t (6.7)	2.47 t (6.6)	2.46 t (6.7)
12	1.22 t (7.0)	1.23 t (7.1)	1.22 t (7.0)	1.23 t (7.1)
ac				
2	2.08 s			
2bute				
2		7.75 dq (9.0, 1.9)		
3		7.65 dq (9.0, 2.2)		
tgl				
3			6.93 dq (1.5, 6.5)	
4			1.51 d (6.5)	
nl				
2	2.49*	2.50*	2.50*	
3	3.89*	3.90*	3.90*	

[a] Abbreviations: Qui = quinovopyranosyl, Glc = glucopyranosyl, Rha = rhamnopyranosyl, dodeca = *n*-dodecanoyl, ac = acetyl, 2bute = 2-butenoyl, tgl = 2-methyl-2-butenoyl, nl = 3-hydroxy-2-methylbutanoyl, and Jal = 11-hydroxyhexadecanoyl. [b] Chemical shifts marked with an asterisk (*) indicate overlapped signals.

The molecular formulas of **9** ($C_{67}H_{114}O_{27}$), **10** ($C_{68}H_{116}O_{27}$), and **11** ($C_{58}H_{102}O_{24}$) were determined by their negative-ion HRESIMS. The positive-ion FABMS of **9**−**11** gave quasimolecular ions $[M + Na]^+$ at *m*/*z* 1373, 1387, and 1205, respectively. The negative-ion FABMS of **9**−**11** showed signals for $[M - H]^-$, in addition to the common fragmentation peaks[4] produced by glycosidic cleavage of the sugar moieties. The ^{13}C NMR spectra of **9**−**11** (Table 4) showed five anomeric signals, and the ^{1}H NMR spectrum of these three compounds (Table 5) showed four doublet methyl signals for 6-deoxyhexose units and signals attributable to the nonequivalent protons of the methylene group at C-2 of the jalapinolic moiety, indicative of the macrocyclic lactone-type structure. The positions of esterification for **9**−**11** were determined by the correlations in the HMBC NMR spectrum. For **9**−**11**, a niloyl group was attached to C-3 of rhamnose″, a *n*-dodecanoyl unit was attached at C-2 of rhamnose′, and a 11-hydroxyhexadecanoyl group was located at C-2 of rhamnose. According to long-range correlations for **9**, a 2-butenoyl group, and for **10**, a tigloyl residue, was located at C-4 of rhamnose″.

Murucins 1−5 were also isolated and characterized by comparison of their physical and spectroscopic data with published data.[4]

Compounds **1**−**6** and **8**−**11** were evaluated in a cytotoxic assay using cultured cells representative of colon carcinoma (HCT-15), cervical carcinoma (UISO-SQC-1), and ovarian carcinoma (OVCAR-5). Compounds **3**−**5**, **10**, and **11** were inactive[5] against all three cell lines (ED_{50} > 5.0 μg/mL). Compounds **1**, **2**, **9**, and **11** exhibited low activity against OVCAR-5 cells (ED_{50} 5.0, 4.2, 4.5, and 4.0 μg/mL, respectively), but were inactive against HCT-15 and UISO cells (ED_{50} > 5.0 μg/mL).

The glycolipids isolated from *I. arborescens* (**1**−**6** and **8**−**11**) are pentasaccharides that differ from the murucins[4] in having

glucose instead of quinovose. In turn, the glycosides obtained from *Ipomoea leptophylla* differ from the arboresins in having the oligosaccharide core branched, and fucose instead of glucose.[6] Pescapreins are branched pentasacharide glycosides from *Ipomoea pescaprae*, with the *n*-dodecanoyl group located in the same position of the arboresins.[8]

Experimental Section

General Experimental Procedures. Melting points were determined on a Fisher-Johns apparatus and are uncorrected. Optical rotations were measured using a JASCO DIP 360 digital polarimeter. IR spectra were recorded using a Bruker model v22. NMR spectra were obtained on a Varian UNITY 400 NMR spectrometer equipped with a 5 mm inverse detection pulse field gradient probe at 25 °C using standard Varian software. Proton and carbon chemical shifts were referenced to internal tetramethylsilane (TMS), with 20 mg of each oligosaccharide being dissolved in ca. 0.75 mL of methyl alcohol-d_4, except for **7**, which was dissolved in D_2O. Electrospray-ionization mass spectra (ESIMS) were recorded on a Micromass model QTOF 2 spectrometer. Positive- and negative-ion FABMS were recorded on a JEOL MStation JMS700 mass spectrometer using *m*-nitrobenzyl alcohol as matrix. The GC-MS system consisted of a HP 6890 gas chromatograph and a HP 5970 mass selective detector in the electron-ionization mode. Silica gel (70-230 mesh, Merck, Darmstadt, Germany) was used for column chromatography. TLC was carried out on precoated Kieselgel 60 F_{254} (0.25 mm thick; Merck, Darmstadt, Germany) plates, and spots were visualized by spraying the plates with 10% H_2SO_4 solution followed by heating. HPLC was performed using a system comprised of an Agilent 1100 binary pump, an Agilent variable-wavelength UV−vis 1100 detector, and Agilent refractive index detector 1100 and a Rheodyne injector.

Plant Material. Samples of *I. arborescens* were collected in San Pedro Huamelula, in the state of Oaxaca, Mexico, in January 2000. Botanical classification was carried out by Biol. J. F. Castrejón, Instituto de Biología, UNAM, and a voucher specimen (No. 963235) is deposited at the National Herbarium (MEXU) in Mexico City.

Extraction and Isolation. Dried and ground roots (600 g) were defatted with hexane at room temperature. The residual material was extracted exhaustively in CH_2Cl_2 to give, after removal of the solvent, a brown solid material (40 g). The brown solid showed two spots by TLC on silica gel, eluted with $CHCl_3$−CH_3OH (9:1) (R_f 0.54 and 0.45), and was subjected to gravity column chromatography over silica gel (500 g) using a gradient of CH_3OH in $CHCl_3$, leading to two chromatographic fractions. Purification of the less polar fraction was carried out by preparative HPLC using a MCH-10 column (10 mm i.d. × 300 mm, 5 μm, Varian), eluting with a mixture of CH_3CN−H_2O (7:3), at a flow rate 1 mL/min at 25 °C, and detection with UV at 215 nm. Compounds **1** (50 mg, t_R 14.0 min), **2** (30 mg, t_R 16.7 min), **3** (50 mg, t_R 20.5 min), **4** (48 mg, t_R 22.2 min), **5** (35 mg, t_R 23.9 min), and **6** (25 mg, t_R 25.3 min) were collected and reinjected until pure. The more polar fraction was purified by preparative HPLC, eluting with a mixture of CH_3CN−H_2O (7:3) at a flow rate 1 mL/min at 25 °C. Compounds **11** (28 mg, t_R 8.2 min), **8** (25 mg, t_R 18.7 min), **9** (30 mg, t_R 20.0 min), and **10** (28 mg, t_R 21.1 min) were purified, in turn, in this manner.

Arboresin 1 (1): amorphous white powder; mp 138−140 °C; $[\alpha]^{25}_D$ −21.0 (*c* 2.0 CH_3OH); IR ν_{max} 3376 (OH), 2985 (C−H), 1735 (C=O), 1450, 1370, 1200, 1090 cm^{-1}; ^{1}H and ^{13}C NMR, see Tables 2 and 3; positive-ion FABMS *m*/*z* 1321 $[M + Na]^+$; negative-ion FABMS *m*/*z* 1297 $[M - H]^-$, 1115 $[1297 - C_{12}H_{22}O]^-$, 1015 $[1115 - C_5H_8O_2]^-$, 853, 707, 561, 433, and 271; HRESIMS *m*/*z* 1297.8365 $[M - H]^-$ (calcd for $C_{63}H_{110}O_{27}$, 1298.8445).

Arboresin 2 (2): amorphous white powder; mp 140−142 °C; $[\alpha]^{25}_D$ −23.1 (*c* 1.9 CH_3OH); IR ν_{max} 3376 (OH), 2985 (C−H), 1735 (C=O), 1450, 1370, 1200, 1090 cm^{-1}; ^{1}H and ^{13}C NMR, see Tables 2 and 3; positive-ion FABMS *m*/*z* 1363 $[M + Na]^+$; negative-ion FABMS *m*/*z* 1339 $[M - H]^-$, 1157 $[1339 - C_{12}H_{22}O]^-$, 1057 $[1157 - C_5H_8O_2]^-$, 1015 $[1157 - C_2H_2O]^-$, 853, 707, 561, 433, and 271; HRESIMS *m*/*z* 1181.8487 $[M - H]^-$ (calcd for $C_{65}H_{112}O_{28}$, 1340.8568).

Arboresin 3 (3): amorphous white powder; mp 140−142 °C; $[\alpha]^{25}_D$ −19.9 (*c* 1.8 CH_3OH); IR ν_{max} 3376 (OH), 2985 (C−H), 1735 (C=O), 1450, 1370, 1200, 1090 cm^{-1}; ^{1}H and ^{13}C NMR, see Tables 2 and 3; positive-ion FABMS *m*/*z* 1377 $[M + Na]^+$; negative-ion FABMS *m*/*z* 1353 $[M - H]^-$, 1171 $[1353 - C_{12}H_{22}O]^-$, 1071 $[1171 - C_5H_8O_2]^-$, 1015 $[1071 - C_3H_4O]^-$, 853, 707, 561, 433, and 271; HRESIMS *m*/*z* 1353.8643 $[M - H]^-$ (calcd for $C_{66}H_{114}O_{28}$, 1354.8724).

Arboresin 4 (4): amorphous white powder; mp 143−145 °C; $[\alpha]^{25}_D$ −20.1 (*c* 2.2 CH_3OH); IR ν_{max} 3376 (OH), 2985 (C−H), 1735 (C=O), 1450, 1370, 1200, 1090 cm^{-1}; ^{1}H and ^{13}C NMR, see Tables 2 and 3; positive-ion FABMS *m*/*z* 1391 $[M + Na]^+$; negative-ion FABMS *m*/*z* 1367 $[M - H]^-$, 1185 $[1367 - C_{12}H_{22}O]^-$, 1085 $[1185 - C_5H_8O_2]^-$, 1015 $[1085 - C_4H_6O]^-$, 853, 707, 561, 433, and 271; HRESIMS *m*/*z* 1367.8879 $[M - H]^-$ (calcd for $C_{67}H_{116}O_{28}$, 1368.8880).

Arboresin 5 (5): amorphous white powder; mp 148−150 °C; $[\alpha]^{25}_D$ −21.8 (*c* 1.9 CH_3OH); IR ν_{max} 3376 (OH), 2985 (C−H), 1735 (C=O), 1450, 1370, 1200, 1090 cm^{-1}; ^{1}H and ^{13}C NMR, see Tables 2 and 3; positive-ion FABMS *m*/*z* 1405 $[M + Na]^+$; negative-ion FABMS *m*/*z* 1381 $[M - H]^-$, 1199 $[1381 - C_{12}H_{22}O]^-$, 1099 $[1199 - C_5H_8O_2]^-$, 1015 $[1099 - C_5H_8O]^-$, 853, 707, 561, 433, and 271; HRESIMS *m*/*z* 1381.8955 $[M - H]^-$ (calcd for $C_{68}H_{118}O_{28}$, 1382.9036).

Arboresin 6 (6): amorphous white powder; mp 148−150 °C; $[\alpha]^{25}_D$ −22.2 (*c* 2.0 CH_3OH); IR ν_{max} 3376 (OH), 2985 (C−H), 1735 (C=O), 1450, 1370, 1200, 1090 cm^{-1}; ^{1}H and ^{13}C NMR, see Tables 2 and 3; positive-ion FABMS *m*/*z* 1421 $[M + Na]^+$; negative-ion FABMS *m*/*z* 1397 $[M - H]^-$, 1215 $[1397 - C_{12}H_{22}O]^-$, 1115 $[1215 - C_5H_8O_2]^-$, 1015 $[1115 - C_5H_8O_2]^-$, 853, 707, 561, 433, and 271; HRESIMS *m*/*z* 1397.8877 $[M - H]^-$ (calcd for $C_{68}H_{118}O_{29}$, 1398.9030).

Arboresinic acid (7): amorphous white powder; mp 148−150 °C; $[\alpha]^{25}_D$ −34.7 (*c* 1.7 CH_3OH); IR ν_{max} 3376 (OH), 2985 (C−H), 1735 (C=O), 1450, 1370, 1200, 1090 cm^{-1}; ^{1}H and ^{13}C NMR, see Table 1; positive-ion FABMS *m*/*z* 1057 $[M + Na]^+$; negative-ion FABMS *m*/*z* 1033 $[M - H]^-$, 871, 725, 579, 433, and 271; HRESIMS *m*/*z* 1033.7730 $[M - H]^-$ (calcd for $C_{59}H_{102}O_{24}$, 1034.7812).

Murucin 6 (8): amorphous white powder; mp 140−142 °C; $[\alpha]^{25}_D$ −19.8 (*c* 2.3 CH_3OH); IR ν_{max} 3376 (OH), 2985 (C−H), 1735 (C=O), 1450, 1370, 1200, 1090 cm^{-1}; ^{1}H and ^{13}C NMR, see Tables 4 and 5; positive-ion FABMS *m*/*z* 1347 $[M + Na]^+$; negative-ion FABMS *m*/*z* 1323 $[M - H]^-$, 1141 $[1323 - C_{12}H_{22}O]^-$, 1041 $[1141 - C_5H_8O_2]^-$, 999 $[1041 - C_2H_2O]^-$, 837, 691, 545, 417, and 271; HRESIMS *m*/*z* 1323.8490 $[M - H]^-$ (calcd for $C_{65}H_{112}O_{27}$, 1324.8574).

Murucin 7 (9): amorphous white powder; mp 135−137 °C; $[\alpha]^{25}_D$ −21.4 (*c* 1.8 CH_3OH); IR ν_{max} 3376 (OH), 2985 (C−H), 1735 (C=O), 1450, 1370, 1200, 1090 cm^{-1}; ^{1}H and ^{13}C NMR, see Tables 4 and 5; positive-ion FABMS *m*/*z* 1373 $[M + Na]^+$; negative-ion FABMS *m*/*z* 1349 $[M - H]^-$, 1167 $[1349 - C_{12}H_{22}O]^-$, 1067 $[1167 - C_5H_8O_2]^-$, 999 $[1067 - C_4H_4O]^-$, 837, 691, 545, 417, and 271; HRESIMS *m*/*z* 1349.8650 $[M - H]^-$ (calcd for $C_{67}H_{114}O_{27}$, 1350.8730).

Murucin 8 (10): amorphous white powder; mp 138−140 °C; $[\alpha]^{25}_D$ −19.4 (*c* 1.9 CH_3OH); IR ν_{max} 3376 (OH), 2985 (C−H), 1735 (C=O), 1450, 1370, 1200, 1090 cm^{-1}; ^{1}H and ^{13}C NMR, see Tables 4 and 5; positive-ion FABMS *m*/*z* 1387 $[M + Na]^+$; negative-ion FABMS *m*/*z* 1363 $[M - H]^-$, 1181 $[1363 - C_{12}H_{22}O]^-$, 1081 $[1181 - C_5H_8O_2]^-$, 999 $[1081 - C_5H_6O]^-$, 837, 691, 545, 417, and 271; HRESIMS *m*/*z* 1363.8808 $[M - H]^-$ (calcd for $C_{68}H_{116}O_{27}$, 1364.8886).

Murucin 9 (11): amorphous white powder; mp 148−150 °C; $[\alpha]^{25}_D$ −20.4 (*c* 2.1 CH_3OH); IR ν_{max} 3376 (OH), 2985 (C−H), 1735 (C=O), 1450, 1370, 1200, 1090 cm^{-1}; ^{1}H and ^{13}C NMR, see Tables 4 and 5; positive-ion FABMS *m*/*z* 1205 $[M + Na]^+$; negative-ion FABMS *m*/*z* 1181 $[M - H]^-$, 999 $[1181 - C_{12}H_{22}O]^-$, 837, 691, 545, 417, and 271; HRESIMS *m*/*z* 1181.7734 $[M - H]^-$ (calcd for $C_{58}H_{102}O_{24}$, 1182.7812).

Acid Hydrolysis of the Chromatographic Fractions. Both chromatographic fractions (50 mg each) were refluxed and separated in 1.0 N HCl (10 mL of water−ethanol) for 1.0 h. The reaction mixtures were taken to pH 5 with NaOH solution, and the solutions were extracted with CH_2Cl_2 and analyzed by GC-MS (25 m × 0.2 mm HP-5 column: He, 1 mL/min; 40 °C, 2 min, 40−250 °C, Δ 15 °C/min, 250 °C 10 min; split 1:40). The less polar chromatographic fraction gave the following acid derivatives: ethyl acetate (t_R 3.0 min), ethyl propanoate (t_R 3.5 min), ethyl butanoate (t_R 4.0 min), ethyl 2-methylbutanoate (t_R 6.6 min), ethyl 3-hydroxy-2-methylbutanoate (t_R 8.65 min), ethyl dodecanoate (t_R 15.0 min), and ethyl 11-hydroxyhexadecanoate (t_R 19.57 min). The more polar chromatographic fraction gave the following acid derivatives: ethyl acetate, ethyl propanoate, ethyl butanoate, ethyl 2-methylbutanoate, ethyl 3-hydroxy-2-methylbutanoate, ethyl dodecanoate, ethyl 11-hydroxyhexadecanoate, ethyl 2-butenoate (t_R 3.8 min), *m*/*z* $[M]^+$ 114 (2), $[M - CH_3]^+$ 99 (28), $[M - C_2H_4]^+$ 86

(10), $[M - OC_2H_5]^+$ 69 (100), 41 (20), 29 (10); and ethyl 2-methyl-2-butenoate (t_R 6.0 min), *m*/*z* $[M]^+$ 128 (5), $[M - C_2H_4]^+$ 100 (54), $[M - OC_2H_5]^+$ 83 (26), 55 (100), 27 (30).

Carbohydrate Analysis. The aqueous phase of the acid hydrolysis reactions was prepared and analyzed by the previously reported procedure,[4] allowing the identification in the more polar chromatographic fraction of α-L-rhamnose (3 mg, t_R 7.9 min), D-quinovose (1 mg, t_R 8.9 min), and d-glucose (1 mg, t_R 15.2 min), while in the less polar chromatographic fraction α-L-rhamnose (2 mg, t_R 7.9 min) and D-glucose (1 mg, t_R 15.2 min) were identified. The sugars were isolated and their absolute configurations were determined by their optical rotations: glucose, $[\alpha]^{25}_D$ +105 (*c* 0.9 CH_3OH); rhamnose, $[\alpha]^{25}_D$ −6.0 (*c* 2.3 CH_3OH); quinovose, $[\alpha]^{25}_D$ +64.0 (*c* 0.8 CH_3OH).

Alkaline Hydrolysis of the Chromatographic Fractions. Both chromatographic fractions (200 mg each) were refluxed and separated in 0.1 N NaOH (10 mL) for 60 min. The reaction mixtures were treated according to the previously reported procedure.[4] The murucinic and arboresinic acids obtained were characterized by NMR and mass spectrometry. The glycosidic acids were refluxed and separated in 1.0 N HCl (5 mL of water−ethanol, each) for 1.0 h. The reaction mixtures were taken to pH 5 with NaOH solution, and the solutions extracted with CH_2Cl_2. The organic layers were washed with H_2O, dried over anhydrous Na_2SO_4, and evaporated. The observed optical rotation ($[\alpha]^{25}_D$ +0.43) was closely comparable to that previously reported[7] for the *S* enantiomer of the ethyl ester of jalapinolic acid ($[\alpha]^{25}_D$ +0.45).

Cytotoxicity Assay. The HCT-15, UISO, and OVCAR-5 cell lines were maintained and treated with various concentrations of the glycolipids, and the cell inhibitory concentrations were determined, as previously reported.[4]

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References and Notes

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